

## CLAIMS

What is claimed is:

1. A method for assaying a first sample for a first probe comprising:

providing a substrate attached to a first target;

5           contacting the substrate with the first sample, wherein the first sample is suspected of comprising the first probe, wherein the first probe comprises a first probe polynucleotide comprising a first tag sequence which does not bind to the first target and a first binding sequence which does bind to the first target, and wherein contacting the substrate with the first sample takes place under conditions in which the first binding sequence can bind to the first

10 target;

               contacting the first sample with a first tag-binding conjugate, wherein said first tag-binding conjugate comprises a first semiconductor nanocrystal conjugated to a first tag-binding polynucleotide, wherein the first tag-binding polynucleotide can bind to the first tag sequence but not to the first target, and wherein contacting the first sample with the first tag-binding conjugate takes place under conditions in which the first tag-binding polynucleotide can bind to the first tag sequence; and

               determining if the first semiconductor nanocrystal is associated with the substrate.

2. The method of claim 1, wherein the substrate is selected from the group consisting of a microsphere, a chip, a slide, a multiwell plate, an optical fiber, a cell, a fixed cell, a tissue, a fixed tissue, a nucleus, and a fixed nucleus.

20           3. The method of claim 1, wherein the substrate comprises a plurality of different targets.

25           4. The method of claim 3, wherein the substrate comprises a microarray.

5. The method of claim 1, wherein the first probe polynucleotide is produced from an amplification process comprising a polymerase chain reaction.

6. The method of claim 1, wherein the first probe polynucleotide is produced from an amplification process comprising contacting the sample with an enzyme having reverse transcriptase activity under conditions in which the enzyme can reverse transcribe RNA to DNA.

5        7. The method of claim 1, wherein the first tag sequence is incorporated into the first probe polynucleotide by employing a first primer polynucleotide that comprises the first tag sequence in an amplification process that produces the first probe polynucleotide.

10      8. The method of claim 7, wherein the first primer polynucleotide binds to the polyadenylate tail of mRNA.

15      9. The method of claim 7, wherein the first primer polynucleotide binds to a plurality of different sequences.

10      10. The method of claim 9, wherein the first primer polynucleotide is degenerate at the four 3' residues and thus comprises a mixture of primers.

15      11. The method of claim 9, wherein the first primer polynucleotide comprises bases at the four 3' residues that can base pair with more than one different base.

20      12. The method of claim 1, wherein the first tag sequence is incorporated into the first probe polynucleotide by ligating a polynucleotide that comprises the first tag sequence to a polynucleotide that comprises the first binding sequence.

25      13. The method of claim 1, wherein the first tag sequence is incorporated into the first probe polynucleotide by adding nucleotides that form the first tag sequence to a polynucleotide that comprises the first binding sequence using terminal transferase.

30      14. The method of claim 1, wherein the first tag sequence is located at or nearer the 5' end of the first probe polynucleotide.

15. The method of claim 1, wherein the first tag sequence is located at or nearer the 3' end of the first probe polynucleotide.

16. The method of claim 1, wherein the first tag sequence comprises a base which is not selected from the group consisting of adenine, guanine, cytosine, thymine, and uracil.

17. The method of claim 1, wherein contacting the sample with the first target takes place prior to contacting the sample with the first tag-binding conjugate.

18. The method of claim 1, wherein contacting the sample with the first target takes place subsequent to contacting the sample with the first tag-binding conjugate.

19. The method of claim 1, wherein contacting the sample with the first target takes place simultaneously with contacting the sample with the first tag-binding conjugate.

20. The method of claim 1, wherein the target is a polynucleotide is located in a metaphase spread of chromosomes.

21. The method of claim 1, wherein the target is a polynucleotide is located in an interphase nucleus.

22. The method of claim 1, wherein the target is a polynucleotide is located in a tissue affixed to the substrate.

23. The method of claim 1, wherein determining if the first semiconductor nanocrystal is associated with the first target comprises applying a light source to the substrate that can excite the semiconductor nanocrystal, and determining if a fluorescence emission from the first semiconductor nanocrystal occurs from the substrate where the target is located.

24. The method of claim 1, wherein the first semiconductor nanocrystal comprises a core selected from the group consisting of ZnS, ZnSe, ZnTe, CdS, CdSe, CdTe, HgS, HgSe, HgTe,

MgTe, GaN, GaP, GaAs, GaSb, InN, InP, InAs, InSb, AlAs, AlP, AlSb, AlS, Ge, Si, Pb, an alloy thereof, and a mixture thereof.

25. The method of claim 24, wherein the core is CdSe.

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26. The method of claim 1, wherein the semiconductor nanocrystal comprises a shell.

27. The method of claim 26, wherein the shell is CdS.

10 28. The method of claim 1, wherein the sample is assayed to determine if the first probe is present in the sample.

15 29. The method of claim 1, wherein the sample is assayed to determine the amount of the first probe present in the sample.

20 30. The method of claim 1, further comprising assaying a sample, which may be the first sample or a second sample, for a second probe comprising contacting the substrate with the sample, wherein the substrate comprises a target which may be the first target or a second target, wherein the sample is suspected of comprising the second probe, wherein the second probe comprises a second probe polynucleotide comprising a second tag sequence which does not bind to the target and a second binding sequence, which may be the same as or different than the first binding sequence, which does bind to the target, and wherein contacting the substrate with the sample takes place under conditions in which the second binding sequence can bind to the target;

25 contacting the sample with a second tag-binding conjugate, wherein said second tag-

binding conjugate comprises a semiconductor nanocrystal, which may be the same as or different than the first semiconductor nanocrystal, conjugated to a second tag-binding polynucleotide, wherein the second tag-binding polynucleotide can bind to the second tag sequence but not to the target, and wherein contacting the sample with the second tag-binding conjugate takes place under conditions in which the first tag-binding polynucleotide can bind to the second tag

30 sequence; and

determining if the semiconductor nanocrystal is associated with the substrate.

31. The method of claim 1, wherein the substrate is washed prior to determining if the first semiconductor nanocrystal is associated with the first target.

5        32. The method of claim 1, wherein a medium is added to the substrate to dilute the concentration of the first semiconductor nanocrystal prior to determining if the semiconductor nanocrystal is associated with the first target.

10      33. The method of claim 1, wherein a plurality of different targets are attached to the substrate, wherein each of the different targets can preferentially bind to a corresponding different probe polynucleotide, wherein the binding of each of the different targets to its corresponding different probe polynucleotide can be separately determined.

15      34. The method of claim 33, wherein the binding of each different probe polynucleotide to its corresponding different target can be separately determined through a different identified position at which each different target is attached to the substrate.

20      35. The method of claim 33, wherein the binding of each different probe polynucleotide to its corresponding different target can be separately determined through the use of a different tag-binding conjugate that binds to each different probe polynucleotide, wherein each different tag-binding conjugate comprises a different semiconductor nanocrystal with different fluorescence characteristics and wherein the hybridization of each different first probe to its corresponding different probe polynucleotide can be separately determined by determining if each different semiconductor nanocrystal is associated with the substrate.

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36. The method of claim 33, wherein the hybridization of each different probe polynucleotide to its corresponding different target can be separately determined by the conditions under which it hybridizes.

37. The method of claim 33, wherein each different probe polynucleotide is bound to a different tag-binding conjugate which comprises a semiconductor nanocrystal the same as the first semiconductor nanocrystal.

5 38. The method of claim 33, wherein each different probe polynucleotide is bound to a different tag-binding conjugate which comprises a different semiconductor nanocrystal.

✓ 39. A first probe polynucleotide assay complex comprising a substrate attached to a first target, wherein the first target is a polynucleotide and is bound to a first binding sequence of a 10 first probe polynucleotide, wherein the first probe polynucleotide further comprises a first tag sequence which is bound to a first tag-binding polynucleotide of a first tag-binding conjugate, wherein said first tag-binding conjugate further comprises a first semiconductor nanocrystal conjugated to the first tag-binding polynucleotide.

315 ✓ 40. A kit comprising:

a substrate comprising a first target, wherein the first target comprises a polynucleotide;  
a first tag-binding conjugate, wherein said first tag-binding conjugate comprises a first 20 semiconductor nanocrystal conjugated to a first tag-binding polynucleotide, and further wherein said first tag-binding conjugate only binds to the first target in the presence of a first probe comprising a first probe polynucleotide comprising a first tag sequence and a first target-binding sequence;

a housing for retaining the substrate and the first tag-binding conjugate;  
instructions provided with said housing that describe how to use the components of the 25 kit to assay a sample for the first probe.

41. The kit of claim 40, further comprising a tag polynucleotide comprising a first tag sequence which binds to the tag-binding polynucleotide and does not directly bind to the first target;  
wherein the housing further retains the tag polynucleotide; and  
30 wherein the instructions further describe how to use the tag polynucleotide to prepare the probe polynucleotide comprising the first tag sequence from the sample.

42. The kit of claim 41, wherein the substrate is a microarray comprising a plurality of targets, wherein the plurality of targets comprises different targets.

5 43. The kit of claim 41, wherein the substrate comprises a plurality of different beads,  
wherein a different target is attached to each of the different beads.

44. The method of claim 1, wherein the target is a polynucleotide located in a cell, which may be fixed or unfixed.